12/05 to assess the number of c-DLI administered and the effect of c-DLI on chimerism. **Results:** Of 135 allo-BMT, 29 DLI were administered to 21 pts (median 1 DLI/pt, maximum, 3), 19 of which were c-DLI and 13 of which were for mini-allo-BMT. A median of 10.5×10^6 CD3+ T cells /kg pt weight (range 5 - 108×10^6) was administered. No unexpected infusion-related toxicities or adverse events were encountered. Eight pts were unevaluable for response, due to full donor chimerism at DLI (4) or insufficient data after DLI (4). Ten pts received 11 c-DLI after min-allo-BMT to improve chimerism (4 NHL; 3 CLL; 2 Acute Leukemia; 1 MM). There were 6 responses in T cell chimerism (> 5% increase) among 5 pts (4 NHL; 1 CLL), 2 to full donor chimerism (2 NHL). The mean increase in chimerism after all c-DLIs was 16% (0 - 50) and among responders the mean increase in T cell chimerism was 24.5% and required a mean 64 days (range 14 – 130 days). Four of 5 responders were in CR before c-DLI and remained in CR, while 4 of 5 non-responders had evidence of disease before and/or after c-DLI (NS by Chi square). Fifteen pts received 21 c-DLI due to persistent or recurrent disease (5 AML, 3 CLL, 3 lymphoma, 1 each CML, MM, MDS, Breast). Two pts were not evaluable for response due to death within 30 days. Three pts had CRs attributable, at least in part, to DLI (1 each AML, CML, MM). **Conclusions:** Prospectively cryopreserved CD3+ donor T cells from mobilized PBSC are frequently employed for DLI after mini-allo-BMT. Thawed c-DLI are readily available when needed and are not associated with unexpected infusion toxicity. C-DLI are frequently effective in improving donor chimerism post BMT, however a prospective comparison of fresh vs c-DLI will be required to assess comparative efficacy.

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THYMOGLOBULIN STIMULATES TH2-TYPE ALLORESPONSES AND PRESERVES REGULATORY T CELL (TREG) FUNCTION IN-VITRO

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Thymoglobulin (rabbit anti-thymocyte globulin) is a potent T cell-depleting agent in-vivo. Nevertheless, its specific effect on T cells remains yet to be fully understood. When tested in-vitro at doses ≥ 500 μg/ml thymoglobulin completely depletes T cells. However, since thymoglobulin level in the serum of treated patients is consistent with a 100 μg/ml concentration in-vitro, we tested this dose in culture with purified T cells alone, or with allogeneic antigen presenting cells (APCs). Complement and 10% human serum were present in culture media. In 12-days long kinetics experiments, stimulation with thymoglobulin on d0, or d0 and d3, or d0, d3 and d6, induced T cell proliferation, measured by 3H-thymidine uptake, with peaks on d 4-5. Thymoglobulin also increased T cell responses when added in primary allogeneic mixed leukocyte culture (MLC). T cells pretreated with thymoglobulin for 3 d prior to MLC were hyporesponsive to alloantigen in secondary MLC and did not show cytotoxic T lymphocyte (CTL) activity, measured by 51Chromium release assay, while proliferated in response to restimulation with thymoglobulin. Supernatants of MLC with responder cells pretreated with thymoglobulin for 3 d contained high levels of IL-13 and IL-5, as opposed to control experiments with responders pretreated with PHA that showed high levels of IFNγ and TNFα. Since after MLC with responders pretreated with thymoglobulin >90% of T cells were CD4+CD25+CD27+ we tested the effect of thymoglobulin on immunomagnetically isolated CD4+CD25+ (regulatory T cells, Tregs) or CD4+CD25- cells. After 5 d of culture with thymoglobulin only CD4+CD25- cells had proliferated and both cell subsets expressed CD25, CTLA4, CD62L and GITR. However, only thymoglobulin-treated CD4+CD25+ cell expressed FoxP3 and suppressed T cell alloreactivity when added to a 3rd party MLC (42±27% inhibition). In conclusion, since we demonstrate that thymoglobulin initially drives T cell alloreactivity to Th2-type responses and preserves Tregs immunosuppressive activity, these findings may prompt new studies in the prevention or treatment of acute GVHD.

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STEM CELL TRANSPLANTATION (SCT) WITH AND WITHOUT CONDITIONING FOR PRIMARY IMMUNE DEFICIENCY (PID) IN CHILDREN: A SINGLE CENTER 25 YEAR EXPERIENCE

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Advances in SCT over the past 2 decades have expanded the spectrum of PID for which this form of curative therapy has been utilized. Failed, delayed and loss of donor engraftment and immune function have emerged as significant long-term problems in children with PID. We report the outcomes of conditioned (COND) and non-conditioned (UNCOND) SCT regimens in PID including: several forms of severe combined immune deficiency (SCID), and non-SCID conditions (Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), and Omenn syndrome) over the past 25 years. The majority of transplants in UNCOND SCID and all UNCOND non-SCID subjects were performed prior to 1999, utilized T cell depleted bone marrow from related donors, and did not include GVHD prophylaxis. The majority of transplants in COND SCID and all COND non-SCID patients were performed in 1999 or later, utilized bone marrow from matched unrelated and related donors and CD34+ selection of apheresed mobilized stem cells from mismatched related donors. Prophylaxis for GVHD was utilized in all recipients of unselected marrow and in recipients of CD34 selected stem cell products with $>5 \times 10^4$ T cells/kg. A total of 55 patients received 75 transplants with overall survivals of 64% and 82% in UNCOND and COND patients respectively. Overall survival in 26 UNCOND SCID patients with related donors was 65% vs 80% in 10 COND SCID patients. Four of 7 UNCOND non-SCID patients survived vs. 7 of 8 COND non-SCID patients. Among SCID survivors, 7, 3, and 1 COND patients and 13, 3, and 1 UNCOND patients received 1, 2, and 3 or more transplants respectively. Among non-SCID survivors all 4 UNCOND and 6 of 7 COND patients received only 1 transplant. Feeding problems and failure to thrive occurred frequently in SCID patients prior to transplantation. Significant pre transplant pulmonary disease adversely affected survival in both COND and UNCOND patients. Fatal post transplant EBV driven lymphoproliferation was seen in only 1 patient, following an UNCOND, T cell depleted bone marrow transplant from a haploidentical, EBV+ sibling. In COND patients, serious post transplant infections were related to central venous catheters, and preexisting pulmonary or GI infection. Our experience suggests that conditioning for SCID and non-SCID children with severe PID results in excellent survival and may prove to offer better long term engraftment and immune recovery.

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IMMUNOPHENOTYPIC AND PROTEOMIC CHARACTERIZATION OF CORD BLOOD (CB) CD56^{BRIGHT} AND CD56^{DIM} NK CELLS: POTENTIAL ROLE OF CB CD56^{DIM} MEDIATING GVL EFFECT

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NK cells are characterized by absent CD3 and expression of CD56 and are classified into CD56^{dim} (90%) that are primarily cytotoxic, and CD56^{bright} that secrete cytokines (Shankaran et al *Nature* 2001). NK subsets carry out their respective functions based on their repertoire of NK receptors (NKRs) (Moretta et al *Annu Rev Immunol* 2001). Despite an immaturity in CB T-cell immunity, there is a similar leukemic relapse rate following UCBT vs. UBMT (Cairo et al *Blood* 1997). Unlike PB NK subsets, there is little information regarding CB NK subset function and NKR expression. We compared PB vs. CB NK subset NKR expression and protein expression. PB and CB cells were positively selected for CD56$^+$ using magnetic beads (Miltenyi) and sorted into CD3$^-$/CD56^{bright} and CD3$^-$/CD56^{dim} and NKR expression measured

(CD16, CD158a {KIR2DL1} CD158a,h {KIR2DL1 and KIR2DS1}, CD158b {KIR2DL2}, CD161, NKG2A, NKG2C, NKG2D, Nkp44, NKp46). 250 μg protein from cell extracts of CB and PB $CD56^{Dim}$ was labeled with isotope of 1 unit of light (PB) and heavy (CB) cleavable ICAT reagent and was subjected to avidin affinity chromatography (Lim et al *Lab Invest* 2004). The labeled samples were digested with trypsin and separated by 3-dimensional liquid chromatography. Peptides were analyzed with tandem mass spectrometry (MS) and searched with SEQUEST TM data base against amino acid sequences in the UniProt protein database. $CD56^{+}$ selection yielded >89% purity (PB-96%, CB-89%). There was no statistical difference (mean ± SEM) in NKR expression between the CB $CD56^{dim}$ and PB $CD56^{dim}$. The PB vs. CB $CD56^{bright}$ had significant increased expression of KIR2DL2 (20.31 ± 2.27 vs. 9.43 ± 0.82, $p < 0.012$) only. The CB $CD56^{dim\ vs.\ bright}$ had increased CD16 expression (85.06 ± 6.75 vs. 40.91 ± 5.74, $p < 0.004$) only. There were 33 and 37 proteins over and under expressed by ≥ 2 fold between CB and PB $CD56^{dim}$ NK cells. Differential function of CB overexpressed proteins were 46% binding, 17% catalytic, 15% signaling, 15% transcription, 3% enzyme, 2% structural, and 2% transport. In conclusion, PB vs. CB $CD56^{dim}$ and PB vs. CB $CD56^{bright}$ had similar NKR expression. The CB vs. PB $CD56^{dim}$ NK cells only had 25% protein expression differences, mostly in catalytic and binding. We hypothesize that CB $CD56^{dim}$ in part contribute to the GVL effect post HLA disparate UCBT similar to PB $CD56^{dim}$ NK cells effect post haploidentical AlloPBSCT.

LEUKEMIA

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FIRST LINE SEQUENTIAL THERAPY WITH INTENSIVE CONSOLIDATION CHEMOTHERAPY AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ASCT) AFTER REDUCED INTENSITY CONDITIONING (RIC) FOR PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA (AML) IN FIRST COMPLETE REMISSION (CR1)

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We reported that RIC-based ASCT can be used in pts with CR1 AML after intensive consolidation chemotherapy (Blaise, Cancer, 2005; Mohty, Leukemia, 2005). In the present analysis, we investigated if this control was maintained after longer follow-up. 37 pts (age: 51 (26-60)) with high risk clinical characteristics (n=26; 70%) (Age ≥ 50: 59%; severe comorbidity: 30%)) and/or poor risk leukemic features (n=24; 65%) (Cytogenetics: 35%; failure of first induction course: 27%; secondary leukemia: 11%; High white blood cell counts: 14%; partial remission: 3%) were included. After CR1, all pts received a low dose cytarabine chemotherapy followed one month later by one course of high dose cytarabine (24 g/m^2) and anthracycline (HIDAC). Pts were then scheduled to receive ASCT after with RIC (fludarabine (180 mg/m^2), busulfan (8 mg/kg), Thymoglobulin (2.5 to 10 mg/kg)) followed with BMT (28%) or PBSCT (72%). It appeared that this schedule was not associated prohibitory toxicity. All following pts were, thus, proposed to receive one month after HIDAC, one course of melphalan (140 mg/m^2) (HDMEL) with auto-SCT followed after recovery by the allo-SCT. Overall, 21 pts received HIDAC only and 16 HIDAC and HDMEL. Median follow-up is 3 years (16 months-70 months). The cumulative incidence (CI) of grade 2-4 aGVHD was 22% (9-35) (Grade 1: 7; Grade 2: 4; Grade 3-4: 4). The CI of cGVHD was 65 % (50-80) (Limited: 10; extensive: 14). The CI of non-relapse death (NRD) was 8% (0-17) (AGVHD: 1; CGVHD: 2). In all, 8 pts relapsed at 5 months (2-19) (CI: 22% (9-35). Relapse was clearly associated with the absence of cGVHD (cGVHD: 4% (4-12), no cGVHD 44% (12-76), p=.02) and at a lesser extent with the intensity of consolidation chemotherapy (HIDAC: 33% (13-53); HIDAC + AUTO; 6% (0-19%), p=.06). 26 pts are still alive in CR1 for overall survival and leukemia-free survival (LFS) probability estimates at 4 years of 67 % (49-81%) and 68% (50-81%) respectively. When restricting the analysis to the 33 pts evaluable for cGVHD, cGVHD remained the only independent risk factor positively influencing LFS (cGVHD: 83% (59-74); no cGVHD (56% (27-81), p=.03). We conclude that RIC Allo-SCT preceded by adequate prior intensive chemotherapy might offer a relatively low NRD while exerting a sustained leukemia control even in high risk pts. The intensity of intensive chemotherapy needed for optimal treatment will be assessed prospectively.

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THE USE OF ADJUNCTIVE LEUKEMIA SPECIFIC THERAPY TO IMPROVE OUTCOME IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TRANSPLANTED USING A REDUCED INTENSITY CONDITIONING (RIC) REGIMEN

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Reduced intensity conditioning (RIC) regimens have increased the proportion of patients eligible for allogeneic SCT. However such regimens are associated with a high risk of early relapse and this represents the major cause of treatment failure. Significantly the use of donor lymphocyte infusions (DLI) as salvage therapy in the first year post RIC allograft is complicated by a high risk of severe GVHD and consequent morbidity and mortality. In order to improve the outcome of RIC allografts there is therefore an urgent requirement for the development of strategies which reduce or, at the very least, postpone the requirement for DLI. We have examined whether the use of leukemia specific adjunctive therapy, in the form of the tyrosine kinase inhibitor Imatinib for the first year post transplant, can be used to improve the outcome of RIC allografts in CML. Patients with CML in first chronic phase and an available sibling donor underwent allogeneic SCT using fludarabine $125mg/m^2$, busulphan 8 mg/kg and alemtuzemab 50 mg. Imatinib was commenced on day +35 and continued until one year post transplant. Escalating dose DLI was administered in patients with evidence of disease relapse after the discontinuation of Imatinib. 23 patients (median age 49 yrs) were transplanted. All patients engrafted promptly and demonstrated mixed T cell chimerism at day+90. The day 100 transplant related mortality was 0%. Two patients developed GVHD (Grades 2 and 3). All patients commenced Imatinib on day +35, 19 of whom tolerated continuous treatment until one year post-transplant. All patients demonstrated a greater than three log reduction in BCR:ABL transcript numbers in the first year post-transplant- 13 achieving molecular remission. After discontinuation of Imatinib DLI was instituted in 12 patients as treatment of molecular or cytogenetic relapse. Of the 10 patients who have completed a course of DLI, 9 have achieved durable molecular remissions. 2 patients developed GVHD in association with DLI. We conclude that the combination of Imatinib and a reduced intensity allograft is well tolerated in patients with CML and may be an effective strategy by which the toxicity of allogeneic transplantation can be reduced without compromising its ability to produce molecular remissions. The use of adjunctive leukaemia specific therapy may provide an effective strategy to improve outcome after RIC allografts in other diseases in which molecularly targeted therapies are available.